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依那普利对CCl_4急性肝损伤大鼠抗氧化功能的影响

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Effect of Enalpril on acute liver injury induced by CCl_4 in rats and its anti-oxidative function

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Abstract

AIM: To investigate the effect of Enalpril on acute liver injury induced by carbon tetrachloride (CCl_4) in rats and its anti-oxidative function.

METHODS: Fifty normal male SD rats were randomly divided into five groups (10 rats/group): Enalpril interventional groups A, B, and C (10, 5, and 2.5 mg/kg, respectively), injury-model group, and control group. Rats in interventional and model groups were given hypodermic CCl_4 (diluted with an equal volume of olive oil). Rats in control group received normal saline injection. Rats with liver injury induced by CCl_4 were then treated with Enalpril (10, 5, 2.5; ig). The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bile acid (TBA) were detected using full automatic biochemical analyzer. Superoxide dismutase (SOD), xanthine oxidase (XOD), glutathione perioxidase (GSH-PX), and malondialdehyde (MDA) were determined using colorimetric method.

RESULTS: Enalpril significantly reduced serum ALT (685 ± 63, 1 241 ± 168, 1 705 ± 83, 2 302 ± 174 nkat/L *vs* 3 531 ± 776 nkat/L in control, A, B, C versus model group respectively; $P<0.01$), AST (1 240 ± 158, 2 430 ± 386 nkat/L *vs*, 3 372 ± 138 nkat/L in control, A versus model group; $P<0.01$, $P<0.05$ respectively), ALP (2 659 ± 248, 2 567 ± 159 nkat/L *vs* 3 609 ± 346 nkat/L in control, A versus model group; $P<0.01$) and TBA (8.48 ± 0.49, 16.35 ± 5.43, 16.92 ± 2.68 μmol/L *vs* 24.16 ± 9.27 μmol/L in control, A, B versus model group; $P<0.01$, $P<0.05$, $P<0.05$ respectively) in acute liver injury induced by CCl_4. The level of XOD in model group was significantly higher than that in control, A, B and C groups (1 042 ± 188 nkat/L *vs* 571 ± 28, 724 ± 18, 821 ± 28, 868 ± 58 nkat/L; $P<0.01$). SOD level in model group was significantly higher than that in control and A group (8 579 ± 861 nkat/L *vs* 6 006 ± 639, 7 135 ± 1 560 nkat/L; $P<0.01$, $P<0.05$). MDA level in interventional group was obviously lower than that in model group and GSH-PX level was obviously higher than that in model group.

CONCLUSION: Enalpril has protective effects for rats with acute hepatic injury induced by carbon tetrachloride and the mechanism closely relates to its anti-oxidative function.

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摘要

目的: 研究依那普利对四氯化碳(CCl_4)所致急性肝损伤大鼠肝损伤和抗氧化功能的作用.

方法: 将50只♂ SD大鼠随机分为5组(每组10只): 药物干预组(10 mg/kg, 5 mg/kg和2.5 mg/kg)、模型组和正常对照组, 药物干预组和模型组均给予皮下注射CCl_4(用等体积的橄榄油稀释), 以制备急性肝损伤的大鼠模型, 正常对照组用等体积的生理盐水注射, 用高(10 mg/kg)、中(5 mg/kg)、低(2.5 mg/kg)剂量依那普利分别对SD大鼠灌胃给药. 全自动生化分析仪测定大鼠血清丙氨酸转氨酶(ALT)、天冬氨酸转氨酶(AST)、碱性磷酸酶(ALP)、胆汁酸(TBA)的活性; 用比色分析法测定超氧化歧化酶(T-SOD)、黄嘌呤氧化酶(XOD)、谷胱甘肽过氧化物酶(GSH-Px)的活性及丙二醛(MDA)的含量.

结果: 依那普利显著降低因CCl_4所致急性肝损伤大鼠血清ALT(正常对照组685 ± 63 nkat/L<10 mg 干预组1 241 ± 168 nkat/L<5 mg 干预组1 705 ± 83 nkat/L<2.5 mg 干预组2 302 ± 174 nkat/L<模型对照组3 531 ± 776 nkat/L), AST(正常对照组1 240 ± 158 nkat/L<10 mg 干预组2 430 ± 386 nkat/L<5 mg 干预组2 788 ± 522 nkat/L<2.5 mg 干预组3 151 ± 917 nkat/L<模型对照组3 372 ± 138 nkat/L), ALP(10 mg 干预组2 567 ± 159 nkat/L<正常对照组2 659 ± 248 nkat/L<5 mg 干预组3 212 ± 198 nkat/L<2.5 mg 干预组3 231 ± 261 nkat/L<模型对照组3 609 ± 346 nkat/L)和TBA(正常对照组8.48 ± 0.49 μmol/L <10 mg 干预组16.35 ± 5.43 μmol/L <5 mg 干预组16.92 ± 2.68 μmol/L<2.5 mg 干

预组17.53 ± 3.59 μmol/L<模型对照组24.16 ± 9.27 μmol/L)的升高. 对急性肝损伤大鼠血清GSH-PX(模型对照组50 ± 54 nkat/L<2.5 mg 干预组 149 ± 111 nkat/L<10 mg 干预组 169 ± 141 nkat/L<5 mg 干预组 170 ± 91 nkat/L<正常对照组 295 ± 194 nkat/L)的活性有明显的升高作用及降低 T-SOD(正常对照组6 006 ± 639 μkat/L<10 mg 干预组7 135 ± 1 560 μkat/L<2.5 mg 干预组 7 538 ± 938 μkat/L<5 mg 干预组 7 589 ± 780 μkat/L<模型对照组 8 579 ± 861 μkat/L)和XOD(正常对照组571 ± 28 nkat/L<10 mg 干预组724 ± 18 nkat/L<5 mg 干预组821 ± 28 nkat/L<2.5 mg 干预组868 ± 58 nkat/L<模型对照组 1 042 ± 188 nkat/L)的含量. 以上均与模型对照组比较[a]P <0.05, [b]P <0.01.

结论: 依那普利的保肝机制和抗氧化作用与其对抗自由基脂质过氧化密切相关.

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0 引言

依那普利具有良好的细胞保护作用, 他通过抑制肝脏受体内血管紧张素转化酶减少肝移植后局部缺血/再灌注的损害[1]. 也可拮抗抗肿瘤药物造成的小鼠心脏和肝脏的毒性作用[2], 在肾炎[3-4]和肾脏移植后[5-7]对肾脏细胞以及心肌细胞[8-11]都具有一定的保护作用. 我们通过CCl_4损伤大鼠模型, 明确依那普利对急性肝损伤的抗氧化功能的影响, 试图阐明其抗肝损伤的可能机制.

1 材料和方法

1.1 材料 ♂ SD 大鼠 50 只, 体质量 250-300 g(购自中国药物生物制品鉴定所动物中心), 随机分为 5 组, 每组 10 只; 分别为正常组, 模型损伤组, 低剂量药物干预组, 中剂量药物干预组和高剂量药物干预组. 除正常对照组外, 各组 SC 500 mL/L CCl_4 橄榄油溶液[2](CCl_4:橄榄油 1∶1, 3 mL/kg, 首次倍量 6 mL/kg).

1.2 方法 将低、中、高 3 个剂量组连续灌胃(依那普利 2.5, 5, 10 mg/kg). 第 5 d 灌胃后 1 h, 模型损伤组和 3 个依那普利干预组同时 SC 500 mL/L CCl_4 橄榄油溶液, 正常组皮下注射相同体积的生理盐水, 每隔 2 d SC 1 次, 共 3 次. 第 12 d 将 5 组 SD 大鼠以颈动脉结扎采血, 肝素锂抗凝全血 2 mL, 全自动生化分析仪测定大鼠血清丙氨酸转氨酶(ALT)、天冬氨酸转氨酶(AST)、碱性磷酸酶(ALP)、胆汁酸(TBA)的活性, 由本院检验科生化室完成; 用比色分析法测定超氧化歧化酶(T-SOD)、黄嘌呤氧化酶(XOD)、谷胱甘肽过氧化物酶(GSH-Px)的活性及丙二醛(MDA)的含量, 试剂盒购自南京建成生物工程研究所; 按操作说明书进行. 肝脏组织以 40 g/L 甲醛液固定, 作常规组织石蜡切片, 进行病理组织检查. HE 染色, 光镜下观察肝脏损伤情况, 肝小叶是否结构完整, 肝细胞有无明显病变.

统计学处理 将所得数据用SPSS 10.0 软件包作 t 检验, 数据以 mean±SD 表示, 计量资料采用方差分析, P<0.05 为差异有显著性.

2 结果

2.1 组织病理学检查 光镜下对照组肝小叶结构完整, 肝细胞无明显异常病变. 模型组 SD 大鼠肝组织损伤明显, 肝细胞出现细胞核固缩, 明显气球样变(重度脂变), 多发融合性坏死, 炎性细胞浸润, 并以小血管周围带为主, 还出现了轻度的纤维增生. 依那普利治疗组, 肝细胞损伤较模型组明显减轻, 肝小叶结构清楚, 仅有少量轻度脂肪变性(图 1, 表 1).

表 1 各组大鼠肝组织病理学检查结果

分组	细胞变性	坏死程度	纤维化
正常对照组	-	-	-
干预组(10 mg)	轻度脂变(30%)	(V)周围少许点灶状坏死	-
干预组(5 mg)	中度脂变(50%)	灶状融合性坏死	-
干预组(2.5 mg)	中重度脂变(70%)	融合性坏死	少许纤维增生
模型对照组	重度脂变(90%)	多发融合性坏死	轻度纤维增生

2.2 肝功能变化 依那普利明显降低 CCl_4 所致急性肝损伤大鼠血清 ALT, AST 的升高, 模型组 ALT, AST, ALP 和 TBA-C 水平较正常组高. (表 2).

2.3 氧化应激状态变化 模型组大鼠血清中 T-SOD 的活性、XOD 含量明显增高, GSH-PX 活性升高, 但差异不显著, 而 MDA 的则无明显变化. 药物干预组在急性肝损伤后血清中 T-SOD 的活性明显比模型损伤组

表 2 各组大鼠血清 ALT, AST, ALP 和 TBA-C 水平比较(mean±SD)

分组	n	ALT (nkat/L)	AST (nkat /L)	ALP (nkat /L)	TBA (μmol/L)
正常对照组	10	685 ± 63[b]	1 240 ± 158[b]	2 659 ± 248[b]	8.48 ± 0.49[b]
干预组(10 mg)	9	1 241 ± 168[b]	2 430 ± 386[a]	2 567 ± 159[b]	16.35 ± 5.43[a]
干预组(5 mg)	9	1 705 ± 83[b]	2 788 ± 522	3 212 ± 198	16.92 ± 2.68[a]
干预组(2.5 mg)	9	2 302 ± 174[b]	3 151 ± 917	3 231 ± 261	17.53 ± 3.59
模型对照组	9	3 531 ± 776	3 372 ± 138	3 609 ± 346	24.16 ± 9.27

[a]P<0.05, [b]P<0.01 *vs* model group.

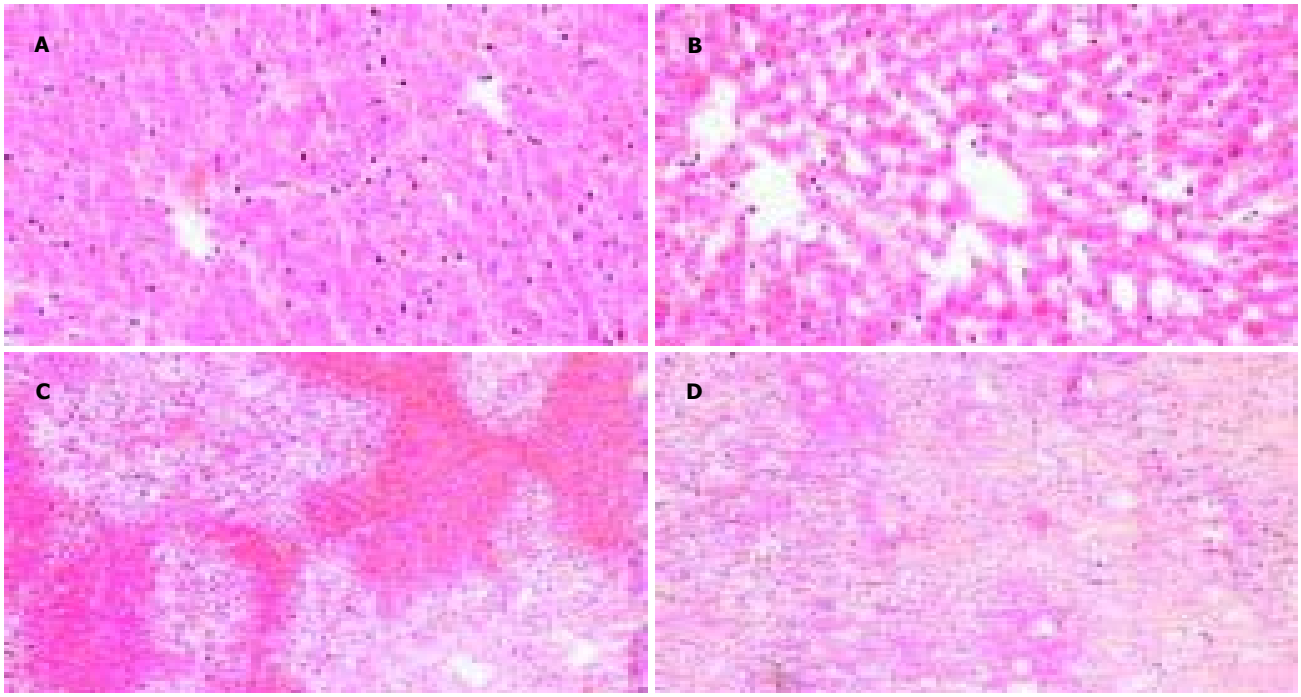

图1 病理学检查结果. A: 正常对照组; B: 干预组(10 mg) – 轻度脂变(30%); C: 干预组(5 mg) – 中度脂变(50%); D: 干预组(2.5 mg) – 重度脂变(95%).

低，二者之间有明显差别; 从XOD的变化可以看出，依那普利治疗后，小鼠肝脏的XOD含量明显低于模型损伤组，而与高低剂量组之间也有明显区别(表3).

表3 各组大鼠血清XOD, T-SOD, MDA和GSH-PX水平比较(mean±SD)

组别	n	XOD(nkat/L)	T-SOD(μkat/L)	MDA(μmol/L)	GSH-PX(nkat/L)
正常对照组	10	571 ± 28[b]	6 006 ± 639[b]	6.49 ± 2.01	295 ± 194[b]
干预组(10 mg)	9	724 ± 18[b]	7 135 ± 1 560[a]	5.56 ± 1.96	169 ± 141
干预组(5 mg)	9	821 ± 28[b]	7 589 ± 780	5.78 ± 1.42	170 ± 91
干预组(2.5 mg)	9	868 ± 58[b]	7 538 ± 938	4.66 ± 0.64	149 ± 111
模型对照组	9	1 042 ± 188	8 579 ± 861	7.19 ± 2.18	50 ± 54

[a]$P<0.05$, [b]$P<0.01$ *vs* model group.

3 讨论

文献报道，CCl_4是一种有毒的环境生物损害剂，在肝氧化代谢中可产生三氯甲基自由基，继而攻击脂质细胞膜，造成肝细胞脂质过氧化性损伤[12-13]. 自由基和脂质过氧化可破坏细胞内钙稳态，引起细胞代谢紊乱甚至死亡. 并使黄嘌呤脱氢酶转化为黄嘌呤氧化酶，加速氧自由基的产生，从而加剧肝损伤[12, 14-15]. 研究表明，肝损伤是多因素参与的复杂过程[16-19]，大量肝细胞受损导致血清ALT，AST显著升高[20-23]. 本实验证实CCl_4可使小鼠血浆转氨酶活力明显上升，同时肝匀浆脂质过氧化产物MDA含量明显增加[24-28]. 说明CCl_4引起肝损伤的机制与脂质过氧化有关. 依那普利能显著降低因CCl_4所致急性肝损伤大鼠血清ALT，AST，ALP和TBA活性升高，表明其具有良好的降酶作用及明显减轻肝细胞损伤程度.我们在模型组大鼠肝脏组织病理切片中观察到，肝小叶索状排列紊乱，肝细胞重度脂变(90% ±)，出现细胞核固缩，并有多发融合性坏死，炎性细胞浸润和轻度的纤维增生. 药物干预组则肝细胞损伤明显减轻，明显降低CCl_4对肝组织的损伤. 说明可减轻肝实质损伤，抑制肝内胶原纤维增生，因而延缓肝纤维化的形成. 本实验中肝损伤模型组，GSH-PX活性升高，但差异不显著，可能依那普利不直接清除自由基，而是通过调节参与体内抗氧化酶的表达来实现的. 研究结果中药物干预组大鼠血清SOD的活性明显升高，而XOD的含量则降低. SOD为高效的清道夫，可抑制自由基启动的脂质过氧化，XOD活性变化表明，由于XOD在黄嘌呤代谢中可以产生氧自由基，依那普利可以降低其活性，减少氧自由基的产生，从而降低细胞的损伤，这也证明依那普利可能是通过调节体内酶的表达来减少氧化损伤的，其本身的氧化还原状态直接影响其活性与功能. 结合以往的研究，推测依那普利在体内能起到抗氧化损伤作用，其保肝机制与其抗自由基脂质过氧化密切相关. 这为今后的研究及应用打下坚实的基础.

总之，依那普利对CCl_4所致大鼠急性肝损伤具有良好的保护作用，其机制在于氧化应激的情况下，通过稳定胞质$[Ca^{2+}]$浓度和调节细胞内抗氧化酶的水平，抑制自由基对细胞的损伤，以达到保护肝细胞和抗氧化作用.

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